SCIENTIFIC ABSTRACT OF THE PROTOCOL

Practical development of gene transfer for cystic fibrosis will likely necessitate aerosol delivery to the lung. This protocol seeks to address two key questions regarding the use of adenovirus vectors for CF gene therapy, can adenovirus vectors be administered safely to the lung at dose levels believed to be required for effective correction of the CF defect, and is aerosolization of vector a safe mode of administration? This protocol is an extension of a previously approved protocol for bronchoscopic administration of a replication-defective adenovirus vector (**Ad2/CFTR-2**) to a lobe of the lung of CF patients. These protocols will be conducted in a staggered, but conccurent manner at the same two institutions. Safety is maximized in this protocol by evaluating two lobar administrations of **Ad2/CFTR-2** prior to initiating the aerosol protocol. We have timed these two protocols such that prior to each aerosol dose, an equal dose and the next higher dose will have been administered by bronchoscope to a lobe of the lung. We will have evaluated the equal dose for two months and the next higher dose for one month.

An earlier study to evaluate the safety of a replication defective adenovirus vector (Ad2/CFTR-1) in the nasal epithelia of CF patients was conducted. In that first protocol (ORDA protocol #9212-036), Ad2/CFTR-1 was administered to four CF patients. In all four cases changes in electrolyte transport were detected following treatment with vector. No adverse effects that could be attributed to the adenovirus vector were detected. A second protocol (ORDA protocol #9312-067), which is ongoing, is designed to address two key questions regarding the use of adenovirus vectors (**Ad2/CFTR-2**) for CF gene therapy, is it safe to administer virus multiple times, and is it possible to obtain evidence of clinical and biochemical efficacy? This second protocol is in its early stages, making it premature to make definitive conclusions about the eventual outcome. However, no results have yet been obtained from the study that would suggest that repeat dosing of adenovirus vectors is unsafe.

The proposed protocol will use the same second generation adenovirus vector, named **Ad2/CFTR-2**, that was used in the second protocol (#9312-067). This type 2 adenovirus lacks E1 and in its place contains a modified transcription unit with the phosphoglycerate kinase (PGK) promoter and a poly A addition site flanking the CFTR cDNA. Results from several studies indicate that PGK promoter will direct modest CFTR expression levels and that it will not be subject to inactivation. The E4 region of the vector has also been modified in that the whole coding sequence has been removed and replaced by ORF6, the only E4 gene essential for growth of Ad2 in tissue culture. The genome of **Ad2/CFTR-2** is 101% the size of wild type Ad2 and it can be grown readily to high titer in culture. Clinical lots of vector are produced using master or working cell bank cells and master virus seed stocks each of which has been subjected to rigorous testing. Vector preparations must pass a series of tests before they are released for clinical use.

This study will evaluate the safety of aerosol administration of **Ad2/CFTR-2** to the pulmonary airway of patients with CF. There will be 8 study groups of 2 patients each, with each patient within a group receiving the same dose of **Ad2/CFTR-2**. The study treatment doses range from 8×10^6 to 2.5×10^{10} IU and increase from the lowest dose to the highest dose in half log increments. The aerosolization protocol is designed to begin after initial safety data is available from the lobar administration protocol. In the concurrent lobar protocol, which uses the same dose increment schedule, 3 patients will receive the virus by bronchoscopic administration to a lobe of the lung. After a 1 month monitoring period and if no adverse effect is noted, 3 patients from the next higher dose group will receive the virus through bronchoscopic administration. After an additional 1 month monitoring period 2 patients from the initial dose group in the aerosol study will receive aerosol administration to the entire lung. A total of 16 patients with mild to moderate disease will be enrolled in the study. Each study patient will be at 90% or more of their peak pulmonary function achieved within the last year. Prior to administration of the virus, study patients will be monitored for at least 3 weeks to ensure that they are clinically stable. Specifically, the patient will not have experienced a CF exacerbation requiring hospitalization or shown evidence of adenoviral shedding within 3 weeks and will not have had an upper respiratory infection within 2 weeks prior to treatment with **Ad2/CFTR-2.**

Each patient will be followed for 21 days prior to treatment, then they will be treated and closely monitored for 28 days post treatment. This post-treatment monitoring includes 3 days as an inpatient, with discharge on the fourth day, and then 24 days as an outpatient. Each patient will be assessed for evidence of an adverse systemic, immune, inflammatory or respiratory response to **Ad2/CFTR-2**. Subsequently, patients will be monitored on a monthly basis for 6 months and then at least every 3 months for 1 year. Long-term follow-up by annual query will continue for at least 10 years. Patient specimens will also be examined throughout the study for any evidence of **Ad2/CFTR-2** viral shedding.

At post treatment Day 7 of the study, each patient will undergo bronchoscopy during which a brushing will be performed. Specimens from the brushing will be evaluated for gene expression by RNA template-specific PCR (RS-PCR). Bronchoalveolar lavage data will be available from patients enrolled in the concurrent bronchoscopic lobar study.

The outcome of this protocol will add further to our knowledge of the safety and efficacy of adenovirus vectors and will be invaluable in the design of subsequent protocols targeted to the respiratory airways and of future generations of adenovirus vectors.